21 An appropriate diagnostic approach can improve detection of CFTR mutations: the experience in Patients with Classical Form of Cystic Fibrosis coming from an Italian Region with high genetic heterogeneity

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Cystic fibrosis is characterized by a very wide mutation spectrum: more than 1600 genetic variations have been described to date.
In some Italian Regions, such as Emilia Romagna (ER), the genetic heterogeneity is particularly high, maybe due to the geographical and historical features. In order to define the Detection Rate (DR) of different diagnostic methods, we analyzed 124 not related patients from ER with a classical form of CF (248 CF alleles). First we performed a commercial RDB analysis searching for 57 common CF mutations, obtaining a DR of about 80%. Then subjects not fully characterized were analysed by DHPLC and direct sequencing: 39 additional CF alleles were identified (15.7%). Among these, the mutation 2184insA was present in 14 chromosomes (5.6%, a high frequency for a mutation different from F508del): all these subjects were from the same town in the east of the region. Another mutation, M1V, was found with a lower incidence (1.2%) only in patients coming from the west part of the region.
Finally, by MLPA analysis a deletion (ex14b-ex17b) was found in three cases (1.2%). Totally we have identified 237/248 CF alleles, 95.6%.
On these bases we have built a diagnostic strategy depending on the indication, the geographic origin and the clinical features of each patient.

22* Clinical phenotype for the G551D mutation

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There is considerable current interest in treating patients with the G551D mutation with drugs that can potentiate its function. However, there are few data on whether the phenotype of patients with compound heterozygocity for G551D differs from patients homozygous for the F508del mutation. The purpose of this study was to compare the clinical phenotype of patients homozygous for F508del and those who are compound heterozygous for G551D, where the second mutation was a severe mutation (Class I, II). Both groups were of similar age 27.7±1.1 y and 26±2.3 y [mean±SD] respectively and there was no difference in age at diagnosis F508del:3.8±1.5 y, G551D:3.9±3.2 y. Patients with the G551D mutation (n = 13) had an FEV_1 of 76±5.8% predicted compared to 56±3.0% predicted in F508del homozygous group (n = 61) ($P < 0.05$). The yearly rate of decline in G551D patients for the previous 3 years was 0.9±0.2% $year^{-1}$, significantly less than the F508del homozygous patients, 1.6±0.1% $year^{-1}$ ($P < 0.05$). There was no significant difference between groups in the percentage colonised with *Pseudomonas aeruginosa* or the use of chronic antibiotic therapy. Fewer G551D patients had pancreatic insufficiency or a diagnosis of CF related diabetes or impaired glucose tolerance. This study suggests that patients with the G551D mutation and a second severe mutation have a milder clinical phenotype than F508del homozygous patients.

23 CFTR gene analysis in the Western-Ukrainian population: an unusually high frequency of the 2184insA mutation

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In Ukraine CFTR gene mutation analysis is performed in two centres: (1) in the capital Kiev and (2) in Lviv – covering mostly the western part the country. Among 132 CF patients treated at Lviv Centre 16 different mutations (p.F508del, p.N1303K, c.CFTRdele2,3(21kb), p.G542X, p.W1282X, c.1898−1G>A, c.2143delT, c.621+1G>A, c.3849+10kbC>T, p.R334W, c.3272−11A>G, c.2721del11, c.1717−1G>A, p.R553X, c.2183AA>G and p.R347H) were found in past, accounting for ~63.7% of all CF alleles. The aim of this study was to identify additional population specific alleles within the frame of the EuroGentest (www.eurogentest.org) fellowship at the Prague CF Centre. Extended mutation analysis was performed by high-resolution melting analysis (HRM), multiplex ligation-dependent probe amplification (MLPA) and direct sequencing. Thus far, we scanned CFTR exons 2, 3, 4, 6a, 7, 11, 12, 15, 16, 17a, 17b, 24, while exon 13 was sequenced. No sequence variations were detected by HRM or MLPA. However, by sequencing we revealed an unusually high prevalence of the c.2184insA mutation since it was found in 16 cases (two were homozygous for this allele). Thus, c.2184insA resides on 6.8% of all CF chromosomes in Western Ukraine and it is the 2nd most common mutation to be included in testing of patients of respective origin. Interestingly, this mutation has been rarely detected among other Slavic CF patients from Russia (1.8%), Poland or Czech Republic (0.18%).
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24 Identification of a novel frameshift mutation in CFTR gene: description, and clinical data

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We report the identification of a novel frameshift mutation 2668del11>ins13 in exon 14a of the CFTR gene. We investigated a male patient with typical CF presentation (disseminated. bronchiectasis, rhinosinusitis, polyposis, pancreatic insufficiency, hepatopathy, colonization by *Pseudomonas aeruginosa*). CFTR mutation analysis in this patient was provided using a multi-step strategy performed in our center: (1) detection of frequent CF mutations by melting point analysis on real-time PCR (F508del, CFTRdele2,3(21kb)G542X, G551D, R553X, N1303K), (2) mutation scoring of uncommon mutations using the commercially available INNO-LiPA CFTR19 and INNO-LiPA17+Tn tests, (3) screening for rare and unknown mutations in the whole coding sequence of the CFTR gene corresponding to the 27 exons and their exon-intron boundaries using a combination of scanning methods DGGE, HRM analysis, sequencing. This approach enabled us to identify the novel frameshift mutation, a deletion of 11 nucleotides in which 5′-GGAACACATAC-3′ is deleted from nucleotide position 2669 and insertion of 13 bp 5′-TCGGTCACAAGAG-3′ at 2669: c.2669_2681del GGAACACATAC ins TCGGTCACAAGAG; p.Trp846fs. The mutation p.Trp846fs is in trans of F508del. The patient CF phenotype would be result of the shift in reading frame, which would produce a non-functional protein. Nevertheless, there is needed a consequential classification of this novel mutation based on its potential for causing disease and their implication for genetic counseling, that is, prenatal diagnosis and carrier testing.